PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
99999-4121		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US00/22619	18/08/2000	18/08/1999
International Patent Classification (IPC) or national classification and IPC A61K48/00		
Applicant UNIVERSITY OF SOUTHERN CALIFORNIA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 11 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☒ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
19/03/2001	02.11.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Julia, P Telephone No. +49 89 2399 8410

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US00/22619

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-32 as originally filed

Claims, No.:

1-23 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

Sequence listing part of the description, pages:

1, filed with the letter of 17.11.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☒ furnished subsequently to this Authority in written form.
- ☒ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

   ☐ copy of the earlier application whose priority has been claimed.

   ☐ translation of the earlier application whose priority has been claimed.

2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

   Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:
   see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	10-15, 17, 20
	No:	Claims	1-9, 16, 18-19, 21-23
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-23
Industrial applicability (IA)	Yes:	Claims	1-17, 19-23
	No:	Claims	18 (see citations and explanations)

2. Citations and explanations
   see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

1. Additional remarks to item II :

The priority document pertaining to the present application was not available at the time of establishing this international preliminary examination report (IPER). Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (19/08/99).

2. Additional remarks to item V :

The present application is directed to non-naturally occurring viral gene therapy vector for cell-specific delivery of nucleic acid to a target cell comprising three structural elements, namely (1) a viral core (comprising at least one viral capsid protein), (2) a functional surface moiety (which can be (i) an immunoprotective element, (ii) a targeting element and (iii) a cell-entry element or combinations thereof) and (3) a linker which can be (i) a multivalent polymer or (ii) a polymer-modified lipid, and wherein said linker associates the two previous structural elements. The claimed vector binds to and delivers the viral core into a target cell and it promotes the production of at least one therapeutic nucleic acid, peptide or protein. The claims refer as examples of (2.1) a synthetic polymer moiety (comprises polyethyleneglycol or a copolymer of glutamic acid with leucine), of (2.ii) a peptide or peptidometric ligand for a cell surface receptor, of (2.iii) a membrane-destabilizing moiety (a copolymer of glutamic acid with leucine or an amphiphilic α-helix which can be derived from the C-terminal domain of a viral env protein, such as residues 598-616 of the Moloney leukemia virus env protein), of (3.i) a copolymer of glutamic acid and leucine amino acids and of (3.ii) a poly-modified lipid modified with a hydrophobic or amphiphilic moiety or with the distal end modified with a ligand or targeting moiety. The application is exemplified by construction of several lipid-polymer-targeting ligand conjugates, such as (i) maleimidoyl-PEG-PE-Cys598-616-MoMuLV, (ii) DSPE-PEG-α-MSH and (iii) DSPE-PEG-rhodamine which are further incorporated in the membrane surface of MoMuLV viral particles or virions.

In view of the general prior art in the field, the IPEA considers that the skilled person was already well-aware that :

1) different liposomes (phospholipid liposomes, lipid vesicles) had successfully been used for encapsulating several (intact) viral particles or virions (Simian virus 40, poliovirus, picornaviruses, rotavirus, Sindbis virus, retrovirus, etc...). These liposomes had been shown to be (i) infectious (including to cells that are normally resistant to virus infection

because of membrane restriction and wherein the efficiency of adsorption has been shown to be dependent on the phospholipid types mixed with the virions, i.e. of the vesicle lipid composition) and to (ii) escape from neutralizing (mono)clonal antibodies, i.e. they were shown to be resistant to the corresponding viral particle or virion antiserum. Thus, the use of a polymer-modified lipid which functions too as an immunoprotective element (functional surface moiety) was well-known in the prior art.

Thus, as far as the linker and the (functional surface moiety) immunoprotective element are not clearly required to be different and both can comprise a (multivalent, synthetic) polymer (a linker/polymer-modified lipid associates the recombinant core with a immunoprotective/polymer-moiety) (see below under "Additional remarks to item VIII"), the IPEA considers that this known prior art directed to liposome-encapsulated virions anticipates at least the subject matter of claims 1-3, 7, 16, 18-19 and 21-22 (Articles 33 (2) and (3) PCT).

2) In this respect, the covalent attachment of an immunoprotective polymer, such as polyethylene glycol (PEG), to the surface of a (replication-defective) recombinant virus was also known in the prior art (PEGylation) with or without the presence of additional polycationic polymers and/or cationic lipids (liposomes). This attachment had been performed by using activated PEG such as TMPEG and this product was known to retain the infectivity and to be protected from neutralizing antibodies both in vitro and in vivo.

3) Moreover, the advantageous presence of additional elements in these liposomes, such as for instance different ligands for targeting the vectors to specific target cells, i.e. presence of targeting elements (and/or cell-entry elements), was known in the prior art too. In particular, being the "targeting element" an antibody (or fragment thereof) having a (high) specificity for a certain cellular receptor, a transferrin, asialoglycoprotein, streptavidin or biotin, etc.... see for instance, A. Fasbender et al., J. Biol. Chem. 1997, Vl. 272 (10), pages 6479-6489 which explicit suggests the introduction of additional functions including a ligand for specific cell-targeting (page 6488, left column). In view that, as pointed out in paragraph (1) above, the claims do not clearly exclude the presence of elements performing different possible functions, such as linker, immunoprotective, targeting and cell-entry, etc... several dependent claims are anticipated by this prior art. In addition, the IPEA considers that several known liposomes can be seen as polymer-modified lipids ("linker") that certainly associate the recombinant viral core with the

targeting element (functional surface moiety).

In particular, such a prior art is considered to anticipate at least the subject matter of claims 1-7, 16, 18-19 and 21-23 (Articles 33 (2) and (3) PCT).

The following documents, concerned with the preparation of polymer-modified lipids (polymer-modified liposomes) comprising different targeting elements, are relevant for assessing the inventiveness of the claimed subject matter : US4948590 using as a targeting element avidin, streptavidin, viral env proteins, etc... (in particular column 18, lines 1-2) and explicitly refers to "retrovirus vectors" but not to virions or recombinant viral core (column 6, lines 23-26). US5631018 using an antibody as a targeting element in immunological protected polymer-modified lipids (among others DSPE-PEG liposomes) (column 10, lines 20-32). Even if this last document only refers to "drug or other pharmaceutical agents" without any reference to recombinant viral core, the IPEA considers that in view of the known prior art using liposomes as delivery agents for different virions (see paragraph (1) above), it would not have required any inventive contribution or skill to use these polymer-modified products for encapsulating (recombinant) viral particles or virions.

4) The attention of the Applicant is also drawn to the fact that as far as the "multivalent polymer" of the linker in claim 1 is not clearly defined (see below "Additional remarks to item VIII"), this "multivalent polymer" comprises (short) sequences of amino acids which certainly "link" or "associate" one polypeptide with another one, i.e. fusion polypeptides or proteins with the presence of peptide linker. Moreover, the direct fusion of two polypeptides or proteins could also be seen as comprising a "linker", namely a (short arbitrary) part of any one of the fusion partners. Thus, in view of this broad interpretation, claim 1 is considered to embrace "non-naturally occurring viral gene therapy vectors for cell-specific delivery of nucleic acid to a target cell" which comprise the recombinant viral core with a chimeric or hybrid envelope protein with or without a linker between the endogenous and the heterologous envelope proteins. In this case, being the "functional surface moiety" a targeting element (heterologous envelope protein) and the "linker" a (multivalent) peptide polymer.

This prior art is considered to anticipate the subject matter of at least claims 1-2, 4, 8-9 and 18-19 (Articles 33 (2) and (3) PCT).

The following documents cited in the International Search Report (ISR) are considered to be relevant :

WO9621036 discloses the production of nucleic acid-encapsulating conjugates (gene delivery vehicles) comprising (i) polycationic polymer (nucleic acid condensing agent) with (ii) an immunoprotective element (PEG) which can comprise additional elements such as (iii) a targeting element and (iv) a cell-entry (endosomolytic agent) and wherein the encapsulated nucleic acid can be a viral vector including a complete viral genome. This document is considered to anticipate the subject matter of at least **claims 1-7, 9-10, 16, 18-19** (Articles 33 (2), (3) PCT). A similar disclosure with a modified viral surface protein with a targeting polypeptide (by linker amino acid residues) is found in document WO9844938 which anticipates at least the subject matter of claims 1-2, 4, 8-9, 18- 19 (Articles 33 (2), (3) PCT).

M.M. Januszeski et al., J. Virol. 1997, Vol. 71, pages 3613-3619 identifies the amphilic alpha-helix peptide of the C-terminal domain of the env protein from Moloney leukemia virus as a destabilizing- membrane agent and thus, as a cell-entry element. WO97/40854 discloses the use of copolymers of glutamic acid and leucine conjugated with additional lipids and targeting elements for delivery of different products into cells (pages 24-25 and 27 as well as figures 3 and 12).

In view of these documents, the considers that the embodiments of the present set of claims which are novel are, however, not inventive in the sense of article 33 (3) PCT.

The attention of the Applicant is also drawn to the fact that the subject matter of **claim 18** is directed to a method for treatment of the human or animal body and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT too. Furthermore, for such a subject matter no unified criteria exist in PCT for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

3. Additional remarks to item VI :

Certain published documents (Rule 70.10 PCT) : WO-A-0002909, publication date: 20.01.00, priority date: 09.07.98, filing date: 08.07.99

4. Additional remarks to item VIII :

The following objections are also raised under Article 6 PCT concerning the clarity of the claims :

i) in respect of **claim 1** the IPEA considers that the wording (**1**) "non-naturally" in particular when referring to "the functional surface moiety" can lead to ambiguous and unclear interpretations (a modified surface moiety, a functional surface moiety not found in the virus, etc...???), (**2**) "for cell-specific delivery of nucleic acid to a target cell" is a purposive-feature or intended use, which does not require, however, any specific additional technical features apart from the ones explicitly referred in the claim. In this respect, the Applicant is reminded that a claim to a substance for a particular use is construed as meaning a substance or composition which is in fact suitable for the stated use; a known product which is per se the same as the substance or composition defined in the claim, but which is in a form which would render it unsuitable for the stated use, would not deprive the claim of novelty (PCT International Preliminary Examination Guidelines, as in force from 09.10.98, Section IV, paragraph III-4.8). (**3**) The "recombinant viral core" is actually found in the description as "recombinant viral particle (nucleocapsid)" or "recombinant core", (**4**) "associates" embraces a large and undefined number of possibilities such as direct association, covalent association, hydrophobic association, etc... even more in view of the broad interpretation of "linker" given in the description (pages 8-9 and pages 23-25) which is not clearly limited to a "multivalent polymer or a polymer-modified lipid" as actually found in claim 1. In fact, "multivalent polymer" embraces any "amino acid linker" too, such as the copolymer of glutamic and leucine referred in several claims. In view of this last interpretation of "linker" the claim would also embrace viral vectors comprising chimeric env, wherein the linker could be a part of the heterologous env sequence (see "Additional remarks to item V" above). (**5**) "promotes the production" could be seen as not encoding and expressing a therapeutic nucleic acid but any factor that enhances/increases production of both endogenous and/or exogenous "therapeutic nucleic acid" and thus, it seems to be a little contradictory with the intended purpose too, namely "for cell-specific

delivery of a nucleic acid to a target cell...". (**6**) it is not clear how to achieve a "cell-specific delivery" if the "functional element" is not clearly defined and/or characterizes as a "targeting element". Thus, a "cell-entry element" does not need to be necessarily "cell-specific".

According to the description it seems to be essential the presence of three different elements so as to (i) evade host's immune system (immunoprotective element), (ii) recognize and bind to specific target cells (targeting element) and efficiently fuse with the target cell and (iii) deliver a transgene (cell-entry element) (page 6 lines 15-20). However, only claim 6 explicitly requires the presence of all three. It is not clear whether the presence of only one (multi-functional?) element, such as for instance the presence of a copolymer of glutamic acid with leucine, can actually perform all three functions, namely as an immunoprotective element, a cell-entry element (membrane-destabilizing moiety) and as a possible linker multivalent copolymer. In this respect, claim 17 refers to a copolymer of a glutamic acid with leucine as being the "immunoprotective element of claim 7, whereas claim 12 characterizes a similar polymer as a "membrane destabilizing moiety" and in claim 20 such a polymer is defined as the linker.

ii) by using the ambiguous wording "derived" in **claim 13**, the scope of the claim is not clear. Actually any amphiphilic α-helix can be obtained/derived from the C-terminal domain of a viral env protein by a suitable type and number of modifications. Moreover this C-terminal domain is not clearly defined (20, 40, 30 residues ???) and in particular the C-terminal domain of **claim 14** which refers to the specific C-terminal domain of the Moloney leukemia virus env protein (there is no amino acid sequence given, see reference on page 27 line 16 in examples).

iii) the use of the wording "essentially" in **claim 20** makes the scope of this claim unnecessarily ambiguous.

iv) the present description does not seem to exemplify all claimed embodiments. In particular, the lipid-polymer-targeting ligands actually exemplified are : (i) maleimidoyl-PEG-PE-Cys598-616-MoMuLV, (ii) DSPE-PEG-α-MSH and (iii) DSPE- PEG-rhodamine which are further incorporated in the membrane surface of MoMuLV viral particles or virions. References are also made to the use of a copolymer of glutamic acid with leucine. However, a product comprising the three different essential elements, namely an

immunoprotective element, a targeting element and a cell-entry element, does not seem to be present in the application. This objection is raised under Article 6 PCT in combination with Article 5 PCT for lack of complete or full technical support of the present set of claims.

v) the attention of the Applicant is drawn to the fact that a non-unity objection will possibly be raised in later stages of the examination (entry into the regional phase) in view of the known prior art and to the fact that there is no clear common technical feature among the different possible constructs. In particular, viral cores or nucleocapsids were already well-known, the presence of a (non-naturally occurring) functional surface moiety was also well-known, the presence of a linker and both the modification of the lipids or virions were also well-known as well as the re-encapsulation with a different functional surface for retargeting the virion.